

PATENT

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Applicants: James M. Musser *et al.*
Serial No.: 08/160,965
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Title : *Streptococcus pyogenes* vaccine

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DECLARATION UNDER 37 CFR § 1.132

GROUP 1800

Sir:

The undersigned, James M. Musser, M.D., Ph.D., hereby declares and states that:

1. I am a co-inventor of subject matter claimed in the above-referenced patent application.
2. I have read the above-referenced application and I have read the attached Amendment, as well as the Office Action to which the Amendment responds.
3. I am an Assistant Professor in the Department of Pathology, Section of Molecular Pathobiology, of Baylor College of Medicine. I have been involved in vaccine research for 4 years. A copy of my curriculum vitae is attached as Exhibit 1.
4. I have been asked to provide evidence that the claimed invention is operative and has patentable utility. In this Declaration, I describe experiments that we have performed which demonstrate that the invention as claimed operates to protect against streptococcal infection. I have also been asked to provide evidence that our specification provides sufficient information to teach one of skill in the art to practice the invention.
5. The following experiments were performed by me and/or under my direction. To test whether the claimed vaccine and vaccination methods protect against lethal group A streptococcal infection, we inoculated mice with cysteine protease purified from *S. pyogenes* strain MGAS 1719. Subsequently, we challenged the mice with *S. pyogenes* strain MGAS

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I hereby certify that this correspondence is being facsimile transmitted
to the Patent and Trademark Office on the date indicated above.

Barbara Rae Venter

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315, which our studies have shown is unusually virulent to mice when administered intraperitoneally (Kapur *et al.*, *Microbial Pathog.*, 15: 327-346, 1993). The protocols we followed are similar to those taught in our specification (*see, e.g.*, pp. 37-38). These experiments are published in Kapur *et al.*, *Microbial Pathog.* 16: 443-450, 1993, which is attached as Exhibit 2.

6. We carried out the study as follows. First, we inoculated male Swiss CD1 outbred mice with either PBS ($n = 10$) or 20 μ g cysteine protease in PBS ($n = 9$) subcutaneously on day 1, followed by intraperitoneal inoculations of the same treatments on days 7, 14, 21, 42, 50, 57, 63, and 79, for a total of nine immunizations. We checked serum anti-cysteine protease antibody levels at days 29, 71, and 84 by ELISA. Then, on day 93, two weeks after the last immunization, we challenged the mice by intraperitoneal injection of strain MGAS 315 as described in our specification (*see, e.g.*, p. 6, lines 23-26). We monitored the mice for survival at one to three hour intervals, recorded their mortality, and plotted Kaplan-Meier survival curves.

7. The results of this experiment, which are shown in the attached Exhibit 3, show that immunization with purified streptococcal cysteine protease conferred significant protection against lethal challenge with the highly virulent MGAS 315 *S. pyogenes* isolate (log rank test; X^2 ; $P < 0.01$). It is also noteworthy that immunization with the cysteine protease also conferred significant protection against *S. pyogenes*-induced early mortality in mice. For example, all 10 mice in the control group were dead by 28 hr post challenge, but only 4 of 9 mice died in the protease immunized group (difference in proportions: z ; $P < 0.003$). Moreover, at the termination of the experiment at 120 hr, 2 of 9 mice in the protease-treated group survived, while none of the 10 mice in the control group survived (difference in proportions; z ; $P < 0.059$). Thus, active immunization with the streptococcal cysteine protease conferred significant protection against lethal group A streptococcal infection.

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8. In a second set of experiments, we have confirmed our earlier finding that vaccination with the purified streptococcal cysteine proteinase confers protection against lethal Group A streptococcal infection in mice. We carried out the study essentially as described in Paragraph 6 above. Briefly, male Swiss CD1 outbred mice were immunized with either PBS (n = 15) or 20 μ g of purified cysteine protease (n = 11 mice) from strain MGAS 1719 for a total of six times (on days 1, 15, 29, 44, 65, and 86). The mice were then challenged by intraperitoneal injection of strain MGAS 315 on day 101, two weeks after the last immunization. We monitored the mice for mortality at 1-3 hour intervals for a total of 157 hours after challenge, recorded mortality, and plotted Kaplan-Meier survival curves. While 12 of the 15 mice in the PBS control group died 40 hours after challenge, only 5 of 11 mice in the group immunized with the cysteine protease had died at the termination of the experiment. These results provide strong confirmation of our earlier findings, and show that immunization of mice with the cysteine protease confers protection to mice against lethal Group A streptococcal infection.

9. Animal tests such as these are reasonably predictive of utility in humans. Beachey *et al.* (*J. Exp. Med.* 150: 862-877, 1979 (attached as Exhibit 4)) provide direct evidence that, for streptococcal peptides in particular, animal tests do accurately predict efficacy of peptide vaccines in humans. For example, Beachey *et al.* state that "[o]ur data indicate that immunization with the adjuvant mixture induces type-specific protective immunity in laboratory animals. We show further that the pep M24 vaccine is well-tolerated in doses sufficient to produce similarly type-specific, primary opsonic and protective antibody responses in man" (p. 863, second full paragraph).

10. It is my belief, as a clinician, that the teachings in our specification enable one of skill to use the streptococcal cysteine protease as a vaccine. Our specification teaches that the streptococcal cysteine protease is an effective vaccine against streptococcal infections in humans, and that the vaccine should be administered in an amount sufficient to confer immunity to Group A streptococcal infection (p. 7, lines 23-25). Now that we have demonstrated that the cysteine protease is effective as a vaccine, the clinician can determine

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the appropriate dosage amount and inoculation regime by following well-established guidelines for peptide vaccines. In this case, the clinician also has the benefit of past experience using a different streptococcal peptide, the M protein, as a human vaccine. For example, Beachey *et al.*, *supra*, used three doses of 200 μ g protein administered subcutaneously every two weeks, followed by a fourth 200 μ g dose 3 4 months after the first dose. Beachey *et al.* noted that these doses are "well-tolerated" and "within conventionally acceptable limits for other vaccines in common use" (pp. 872-873, bridging sentence). Once we had taught that the streptococcal cysteine protease is an effective vaccine, the clinician would reasonably expect that the dosage regime used for M protein would likewise be effective when using the cysteine protease.

11. Similarly, our specification, coupled with conventional guidelines and experience using the streptococcal M protein as a vaccine, provide the clinician with knowledge as to an appropriate vaccine formulation. Our specification teaches that the active ingredient in the vaccine formulation is the streptococcal cysteine protease (see, e.g., p. 7, lines 1-8). It is within conventional knowledge that peptide vaccines are normally administered in conjunction with an adjuvant. Experience using the streptococcal M protein as a vaccine provides particular examples of suitable adjuvants. For example, Beachey *et al.*, *supra*, published a protocol for vaccinating humans using the streptococcal M protein. Beachey *et al.* used aluminum hydroxide gel as the adjuvant in their protocol because "aluminum-hydroxide gels have been well-tolerated in man and have been shown to serve as good adjuvants for the stimulation of the immune responses to other vaccine antigens" (p. 866, second paragraph). Thus, one of skill would expect that aluminum hydroxide would be an effective adjuvant for the cysteine protease.

12. Our patent specification also provides the clinician with a simple means to determine empirically an effective dosage regime. For example, Example 16 (pp. 28-29) describes use of an ELISA for measuring antibodies against the cysteine protease. Similarly, Beachey *et al.*, *supra*, used an ELISA to evaluate the effectiveness of their streptococcal M

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protein vaccine (p. 865). Using this test, Beachey *et al.* found within six weeks that doses of 100 μ g were not effective at inducing an immune response. They simply increased the dose to 200 μ g, which they found was effective within two weeks (p. 867, last paragraph). This illustrates the ease and quickness with which the clinician can evaluate the effectiveness of a streptococcal peptide vaccine. Of course, once one has performed this experiment on one fairly small group of humans to determine the effective dose, this same dosage would be used for all future vaccines.

DECLARATION

I declare that all statements made here of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: _____

James M. Musser, M.D., Ph.D.

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